THE INFLUENCE OF GENERAL ENVIRONMENTAL CONDITIONS ON THE PERIODICITY OF ENDOMIXIS IN PARAMECIUM AURELIA.

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(Twelve figures.)

It is clear from the evidence submitted in previous papers that there are normal, minor, periodic fluctuations (rhythms) in the rate of reproduction of *Paramecium*,[1] and that at the low point in the division rate between two rhythms there normally occurs an intracellular reorganization process (endomixis).[2] The data previously presented also show that in *Paramecium aurelia* endomixis occurs, generally speaking, at intervals of about four weeks or about fifty generations, and that the synchronism of the process in different lines of the same race under identical conditions is remarkably exact.[3] But, as was stated incidentally, on the basis of our experience in working out the cytological phenomena of endomixis: "It is possible to retard or hasten the occurrence of the process by the character of the culture medium. For example, it may occur a few days earlier in animals not supplied daily with fresh culture fluid than in the regular lines."[4] Such being the case it is important to determine the influence of environmental conditions on the duration of the rhythms and the occurrence of endomixis.

The present paper comprises chiefly a study of the effects of what may be termed general changes in the culture conditions,

[1] Woodruff and Baitsell, "Rhythms in the Reproductive Activity of Infusoria," *Journ. Exper. Zoöl.*, XI., 4, 1911.

[2] Woodruff and Erdmann, A Normal Periodic Reorganization Process without Cell Fusion in *Paramecium*," *Journ. Exper. Zoöl.*, XVII., 1914; Erdmann and Woodruff, The Periodic Reorganization Process in *Paramecium caudatum*," *Journ. Exper. Zoöl.*, XX., 1916; Woodruff, "Rhythms and Endomixis in Various Races of *Paramecium aurelia*," BIOL. BULL., XXXIII., 1917.

[3] Cf. Woodruff and Erdmann, 1914, Tables 1, 2, 3.

[4] Woodruff and Erdmann, 1914, p. 485.

such as markedly different culture media and temperatures on rhythms and endomixis. It seemed important to obtain a more definite background of knowledge of the influence of what perhaps may be called normal environmental changes before attempting to study the influence of, for example, specific chemical agents on the process.

It is assumed in the present paper that the reader is familiar with the earlier work on *Paramecium* which has been published from the Yale Laboratory.

Material and Methods.

The organisms employed in the work were from pedigreed cultures of *Paramecium aurelia*. Some of these had been under culture conditions for long periods—one for more than 5,000 generations—while others were started with this work in mind. Each of the five races used was started originally with a 'wild' individual which was secured from a locality far removed from that of the others, so that representative diverse material was afforded. The early life history of each of these cultures has been presented in connection with other work and the reader is referred to these papers for further details.[1]

All the organisms studied have been carried in pedigreed subcultures isolated from the respective main cultures of the various races, and since the method of conducting such cultures has been described many times in earlier papers it need not be repeated in detail here. Suffice it to say that the method involves the isolation of one or more animals from each line of every subculture practically every day and in addition, for the work in hand, the preservation and cytological study of some of the stock animals left over at the time of the daily isolations. In this way the occurrence of endomixis has been determined.

The main cultures have been carried on the 'varied' culture medium which we have found for ten years so favorable in breeding *Paramecium*.[2] This consists of infusions of vegetable and animal debris collected from time to time from laboratory

[1] Cf. especially Woodruff, Biol. Bull., XXXIII., 1917.

[2] Woodruff: "The Life Cycle of *Paramecium* when Subjected to a Varied Environment," *American Naturalist*, XLII., 1908.

aquaria, ponds, etc., and, of course, thoroughly boiled and allowed to attain room temperature before being used. Some of the subcultures directly involved in the experiments have been bred on this medium. Others have been bred on the beef-extract medium which we have employed in other work on *Paramecium*,[1] or on other media which will be described in connection with the individual experiments.

The subcultures which have been the basis of the present work may be tabulated as follows:

SUBCULTURES.

A (from Main Culture I) Oct. 15, 1914, to Feb. 12, 1916. (485 days.)
4675th to 5592d generations.

AE (from IE)[2] Oct. 15, 1914 to Aug. 12, 1915. (Twice restarted during the 300 days.)
4637th to 5079th generations.

O (from III) Oct. 15, 1914 to Nov. 20, 1915. (Once restarted during the 400 days.)
17th to 775th generations.

B (from IV) Jan. 8, 1915 to Jan. 14, 1916. (372 days.)
3d to 609th generations.

M (from V) July 17, 1915 to Feb. 23, 1916. (222 days.)
3d to 550th generations.

W (from VI) Aug. 12, 1915 to Jan. 14, 1916. (152 days.)
3d to 302d generations.

The data are presented chiefly by graphs of the division rate of the various subcultures. These are plotted by averaging the daily rate of division of the several lines of the respective subcultures and then again averaging this for five-day periods. The figures 1, 2, 3 represent divisions and 10, 20, etc., indicate the number of the five-day periods. An *E* shows that endomixis was observed during the five-day period. Inclusion of a part of the curve within brackets indicates that the cells were not studied cytologically during this time. Cf. Figs. 1 and 7. Since so much depends on these graphs and the five-day periods which they comprise it may be well to repeat a statement made in a previous paper:[3]

[1] Woodruff and Baitsell, "The Reproduction of *Paramecium aurelia* in a 'Constant' Culture Medium of Beef Extract," *Journ. Exper. Zoöl.*, XI., 1, 1911.

[2] *IE* is a subculture isolated from Main Culture I. in October, 1913.

[3] Woodruff and Erdmann, *Journ. Exper. Zoöl.*, 1914, p. 477.

"The five-day period was adopted in the presentation of our results because this was the method of constructing the

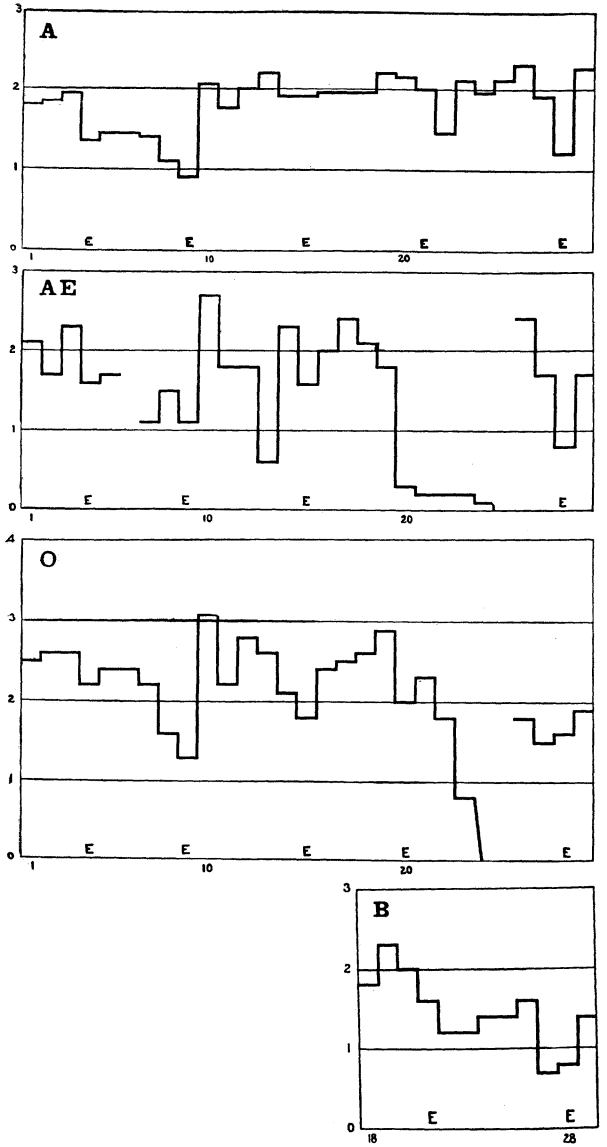

FIG. 1.

graph emphasized in the original study of rhythms in this culture.[1] It is realized, of course, that a five-day period is

[1] Woodruff and Baitsell, *Journ. Exper. Zoöl.*, XI., 1, 1911.

largely an arbitrary one and that the ideal graph would present the momentary changes in the metabolism of the cell. Data for such a curve being absolutely impossible to secure, it might seem at first glance that the daily record of division would approach most nearly to this ideal condition. As a matter of fact, the twenty-four-hour period is as arbitrary as the five-day period when it is considered that this is a long period when compared with the metabolic changes in the cell and that the daily record, made at approximately 11 A.M., would merely give the divisions actually completed during the previous twenty-four hours. For example, let us assume that, at the time of isolation, two animals are present, representing one division during the previous twenty-four hours. The record for that day is one division. One animal is then isolated and it divides within an hour and each of the resulting cells again divide twice before the next isolation. The record for this second day is three divisions, thus the record for the two days shows a different division rate for each day, *i. e.*, one division against three divisions, whereas a more true, but not a perfect, picture of the state of affairs is given by the statement that four divisions occurred in forty-eight hours. One might follow this argument to its logical conclusion and assume that the best method of presentation would be to average for considerable periods, *e. g.*, 10 or 30 days, but this obviously would tend to obliterate any fluctuations in the rate which are not of relatively long duration. The adoption of the five-day period was made in recognition of both of these contingencies, and it was of a duration particularly well suited to show the effect of the process on the reproductive rate, because the process extends over about nine cell divisions or a period of about six days. Consequently the effect of the process makes itself evident in the five-day plot. Certain apparent irregularities in the coincidence of the phenomena are, from an actual study of all the data at hand, clearly due to the fact that the five-day period is not ideal."

EXPERIMENTS—SERIES I.

The experiments of Series I. may be outlined as follows:

A. Study of the periodicity of rhythms and endomixis in

different races of *Paramecium aurelia* when bred under the same *varied* culture conditions.

B. Study of the periodicity of rhythms and endomixis in

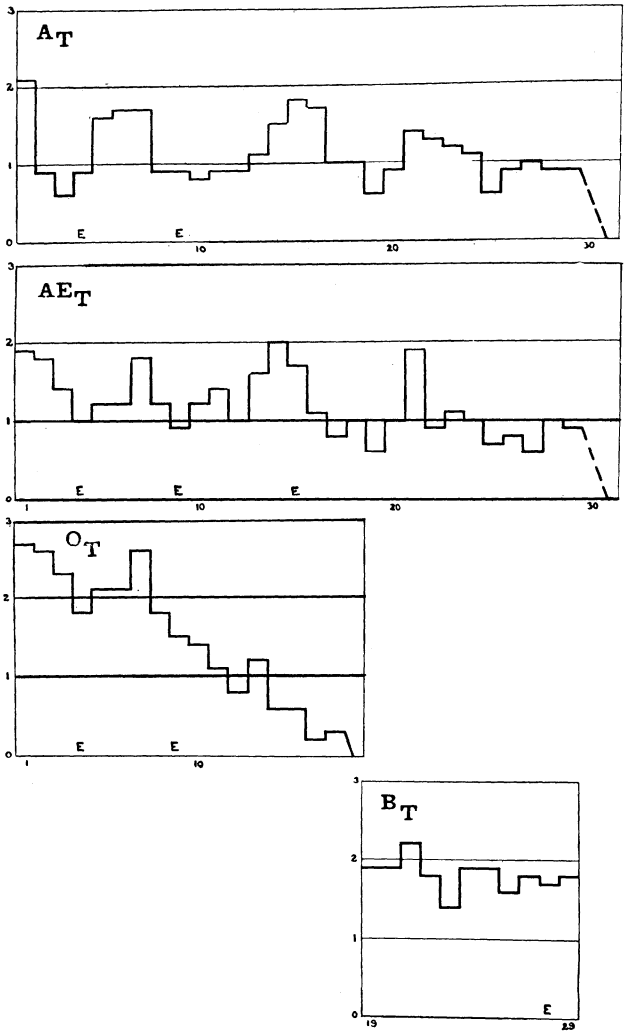

FIG. 2.

different races of *Paramecium aurelia* when bred under the same *constant* culture conditions.

C. Study of the periodicity of rhythms and endomixis in the *same* races of *Paramecium aurelia* when bred under the *varied* and under the *constant* culture conditions.

These experiments were carried on from October 15, 1914, to March 10, 1915; a period of 145 days. The subcultures employed were *A*, *AE*, and *O* throughout the work and *B* from its isolation on January 8, 1915, to the end. Each of these subcultures represents a different race of *Paramecium aurelia*, except *A* and *AE*, both of which were originally derived from the same stock, Main Culture I., about 1,000 generations before.

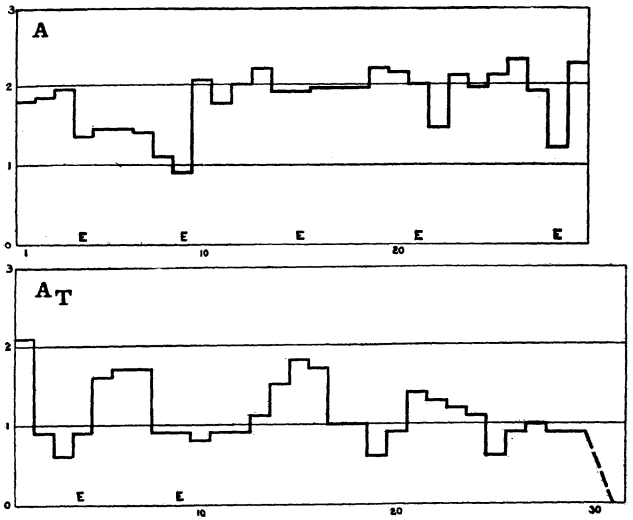

FIG. 3.

All of these subcultures were subjected to a varied culture medium and to the ordinary fluctuation in room temperature.

Sister subcultures (designated *At*, *AEt*, *Ot* and *Bt*), were isolated line by line from the above cultures at the start of the experiment and were subjected to a constant culture medium of beef extract. The temperature was maintained relatively constant at about 26° C. in a thermostat.

A.

Study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred under the same *varied* culture conditions.

Fig. 1 presents the graphs of the division rate of subcultures *A*, *AE*, *O* and *B* throughout this experiment. A study of the

figure shows that *A* underwent endomixis at periods Nos. 4, 9, 15, 21 and 28. *AE* showed endomixis at period 4 and then was lost by an accident. A new *AE* was started by isolating line by line from *AEt* (which had been branched from it 25 days before and subjected to the constant culture conditions).

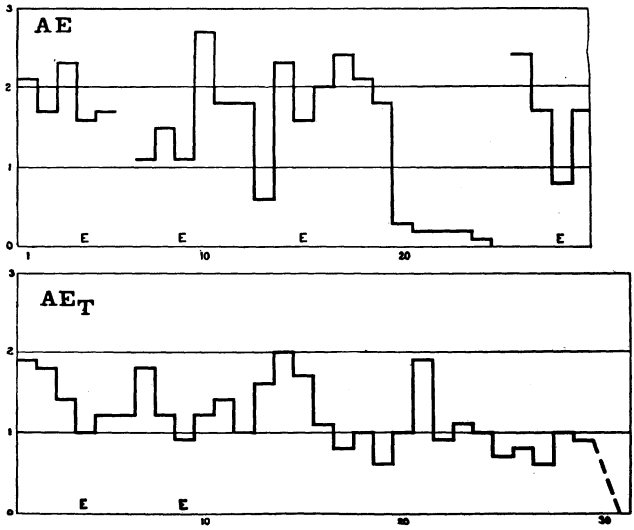

FIG. 4.

AE then showed endomixis at the periods 9 and 15 and died out in period 25 without again undergoing endomixis. Still another *AE* was isolated immediately from *AEt* and had endomixis during the 28th period. A similar survey of the graph of *O* shows that endomixis took place at periods 4, 9, 15 and 20. Subculture *O* died at period 25 from causes unknown. It was restarted from the main culture of this race at once and had endomixis at period 28. Subculture *B* was begun in period 18, that is immediately on the isolation of the race from 'wild' material. Endomixis occurred at periods 21 and 28.

Tabulation of these data shows that endomixis occurred in these subcultures as follows:

A at periods.....	4	9	15	21	28
AE " "	4 lost	9	15	0 died	28
O " "	4	9	15	20 died	28
B " "	–	–	—	21	28

This experiment, in which different races were bred under the same varied culture conditions, shows an almost perfect synchronism of endomixis in all the races; that is, in *A* bred throughout without interruption on the varied medium; in *O* bred until its death near the end of the experiment on the same medium and again in the new *O* isolated from the main culture; in *B*, which was isolated from 'wild' material during the progress of the work; and finally in *AE*, which was restarted twice from a sister culture bred under "constant" environmental conditions.

The most reasonable conclusion, on the basis of this experiment, to account for the fact that the different races immediately showed endomixis synchronously, is that the general culture conditions initially influenced the appearance of the process; that is, brought about its consummation a few days earlier or later than it would have appeared under the former environment of the races, and that, once established, the rhythmic period characteristic of the species persisted and maintained the synchronism of endomixis. If this conclusion is justified then it must be assumed that the synchronism of *B* is due to the chance isolation of this race at just the same period in the rhythm which *A*, *AE* and *O* were at at the time, or so near this period that the environmental change immediately made it coincide with that of the other races. The alternate hypothesis would be that there is in all races of this species a definitely established synchronism which holds under all normal environmental conditions. But such a theory would require more than one series of experiments to render its discussion profitable!

B.

In this set of experiments a study was made of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred under the same practically constant culture conditions, as already described. The results are shown graphically in Fig. 2.

Subcultures *At* and *AEt* (both derived from the same race, I, about 1,000 generations previously) showed endomixis in preserved specimens at periods 4 and 9 and *AEt* also at period 15. Neither culture again underwent the process during a

period of over 100 days before it died, though the characteristic 'rhythms' in the division rate are apparent during this time. It is possible that the process did occur at about periods 19 and 25 (cf. Fig. 2) and was overlooked, but I believe that this is highly improbable in view of the thoroughness of the search.

Subculture *Ot* underwent endomixis at periods 4 and 9 and then died without repeating endomixis at the next expected period (cf. Fig. 2).

Subculture *Bt*, as the graph shows, had endomixis at the 28th period, just before the experiment was concluded. From the character of the curve it would be expected at period 23, but it was not observed.

The endomictic periods of the four subcultures (3 races) of *Paramecium* in this experiment may be tabulated as follows:

At at periods.....	4	9	0	0 died
AEt " "	4	9	15	0 "
Ot " "	4	9	0	– "
Bt " "	–	–	–	28

These experiments show three points of considerable interest. In the first place they corroborate, for races bred under constant culture conditions, what was found in the same races when bred under varied culture conditions in the experiment already described. That is, *At*, *AEt* and *Ot* show a perfect synchronism of endomixis, and this is most reasonably explained by assuming that the general culture conditions, at least initially, influenced the appearance of endomixis and that, once established, the rhythmic period characteristic of the organism persisted. *Bt* affords no data for comparison with the other races of this set of cultures, as they had ceased to show endomixis before *Bt* was started.

A second point of importance is that the 'rhythms' in the division rate are to a certain extent independent of endomixis—that is of the definitive series of nuclear phenomena—because the rhythms persist for a while in the absence of the morphological changes. One may suggest that the rhythms in the culture are an expression of the physiological conditions antecedent to the definitive onset of the nuclear changes—in other

words that the cell has undergone the preliminary stages of endomixis which ordinarily call forth the observable nuclear changes but that in the cases in hand the latter were never realized. This is equivalent to making the term endomixis coextensive with the term rhythm—the term rhythm denoting the physiological effect as indicated in the reproductive activity, while the term endomixis covers all the underlying physiological

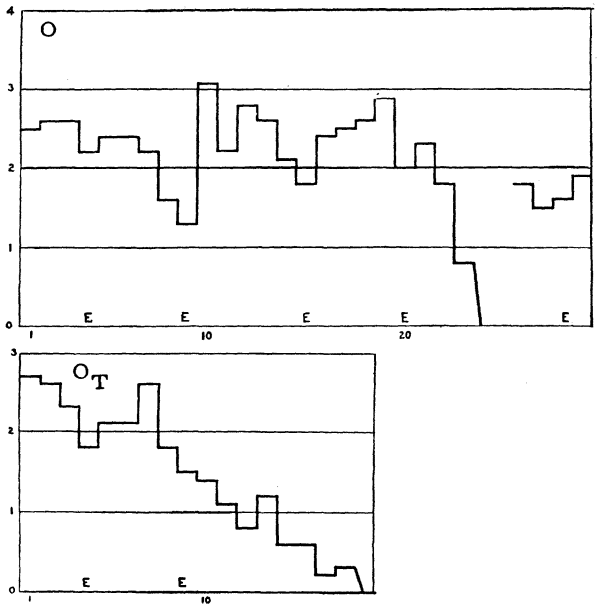

FIG. 5.

changes together with the definitive nuclear phenomena characteristic of the low point in the division rate. The term endomixis was *not* used in that broad sense when first employed by Woodruff and Erdmann and it is unprofitable to extend it now. Applying the term strictly to the complicated reorganization process of the cell does not deny the undoubted fact that these changes are but the expression of the climax of a series of physiological phenomena which probably extend back to the consummation of the previous endomictic period. So, it seems to be a more or less academic question whether rhythms and endomixis are independent. Certainly the rhythms occur for a while without endomixis in *sensu stricto*—but in all such cases the culture has died before very long.

This leads to the third point of interest which is the death of the cultures after the cessation of endomictic phenomena. This is true in each instance: *At*, *AEt*, and *Ot* (cf. Fig. 2). The data from these experiments perhaps are not sufficiently extensive to make sure that this is not a case of *post hoc sed non propter hoc*, but they make it highly probable that endomixis is necessary for the continued life of the race.

C.

The data which have been presented in the study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred under varied and under constant culture conditions, may now be analyzed from the point of view of the periodicity of these phenomena in the *same* races under varied and under constant culture conditions, since experiments *A* and *B* of this series were conducted simultaneously.

This analysis is readily made by a study of Figs. 3, 4, 5 and 6, which consist of a combination of the graphs already presented from the other point of view. The graphs are paired, one above the other, so that identical five-day periods coincide. For example, in Fig. 3, period 10 of *A* is directly above the same period of *At*. Since the endomictic periods of all these cultures have just been considered, it is only necessary to tabulate them for reference:

A underwent endomixis at periods....	4	9	15	21	28
At " " " "	4	9	0	0	0 died
AE " " " "	4	9	15	0 died	28
AEt " " " "	4	9	15	0	0 died
O " " " "	4	9	15	20 died	28
Ot " " " "	4	9	0	—	died
B " " " "				21	28
Bt " " " "				0	28

From this table it is apparent that the synchronism of endomixis is practically perfect in the *same* races when bred under *different* environmental conditions. The first two experiments showed that it was the same for *different* races when bred under the *same* environmental conditions whether varied or constant. Therefore this experiment corroborates and broadens the conclusions derived from the former ones and shows clearly

that the periodicity of endomixis is largely independent of the character of the culture medium—the general environmental conditions—within the rather wide limits in which it has been varied in parts *A*, *B* and *C* of this series of experiments. At most the culture conditions initially influence the appearance of endomixis. In other words, the organism is set, so to speak, to undergo endomixis approximately once a month and this it does under any more or less favorable environmental conditions. A sudden change, however, of these conditions may bring about endomixis slightly earlier than it otherwise would have occurred but after this the usual rhythmic period of the species is maintained.

Experiments—Series II.

The experiments of this series may be outlined as follows:

A. Study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *large* amount of culture medium supplied fresh *daily*.

B. Study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *small* amount of culture medium changed on *alternate days*.

C. Study of the periodicity of rhythms and endomixis in the *same* races of *Paramecium aurelia* when bred in a relatively *large* amount of culture medium supplied fresh *daily*, and in a relatively *small* amount of culture medium changed on *alternate days*.

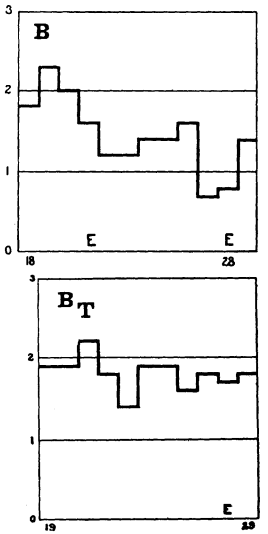

Fig. 6.

These experiments were begun on July 2, 1915, and continued until February 22, 1916. Five different races of the organism were employed. Three of them (*A*, *O*, and *B*) were the same subcultures which were used in the experiments of Series I., and which had been continued during the interim—that is, from March, 1915, to July, 1915. Therefore the numbering of the five-day periods was continued from the earlier work and the

first period of the present series is number 53. Two new races were secured from diverse localities as already described and subcultures from these were begun and made a part of this experiment at once. These subcultures were designated *M* and *W*.

All these subcultures were supplied daily with the regular varied culture medium. Sister subcultures (designated *As*, *Os*, *Bs* and *Ms*) were isolated line by line from the above-mentioned subcultures at the start of the work and were subjected to the same varied culture medium but the amount was reduced to one half that supplied to the parent subcultures and the medium was changed at intervals of 48 hours instead of 24 hours.

It has been shown in previous papers,[1] that the rate of reproduction of *Paramecium* is markedly influenced by the volume and the freshness of the culture medium. This was found to result from the accumulation of the excretion products, in view of the fact that a medium which contains the excretion products of a heavy growth of paramecia has a decidedly depressing effect on the division rate of this organism.

Accordingly it seemed that the excretion products of *Paramecium* afforded the most natural means of quickly modifying the division rate in order to determine the effect of this on the rhythms and endomictic periods. Obviously the experimental conditions involve two variables—excretion products, and their effect, the lowering of the fission rate—so that the specific influence of one or the other cannot be determined from the data given below. But that is not of importance in the present work which is merely an endeavor, as already stated, to determine the effect of normal environmental changes.

A.

This set of cultures was carried on in order to study the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *large* amount of varied culture medium supplied fresh *daily*. The experi-

[1] Woodruff, "The Effect of Excretion Products of *Paramecium* on its Rate of Reproduction," *Journ. Exper. Zoöl.*, X., 1911; Woodruff, "The Effect of Excretion Products of Infusoria on the Same and on Different Species, with Special Reference to the Protozoan Sequence in Infusions," *Journ. Exper. Zoöl.*, XIV., 1913.

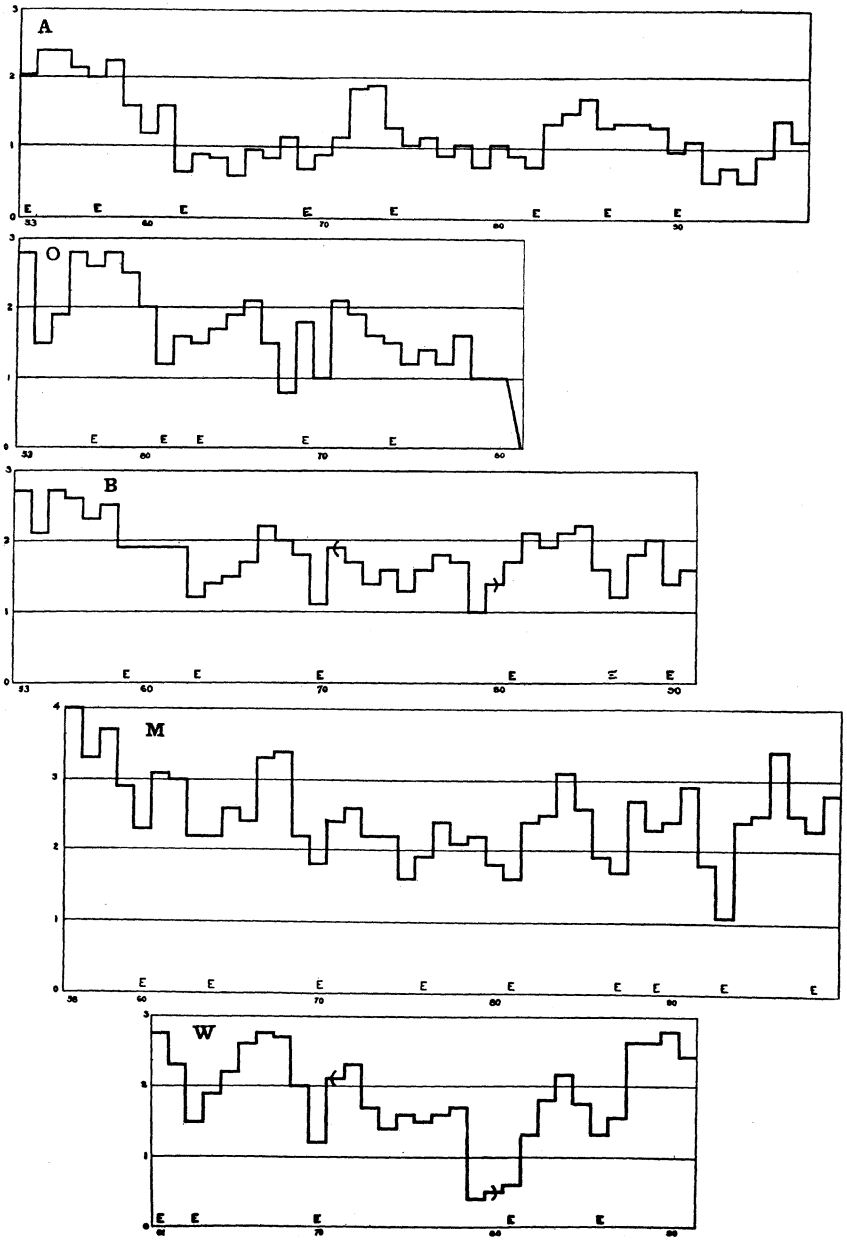

FIG. 7.

ment is essentially a repetition of experiment *A* of Series I (cf. p. 443) since three races (*A*, *O* and *B*) and the character and amount of culture medium are the same as employed before. The results, therefore, now will be analyzed from the viewpoint of Series I. and later from that of the present series.

The results are shown graphically in Fig. 7 from which it is evident that:

A underwent endomixis at periods . . .	53	57	62	69	74	82	86	90					
O " " " " . . .		57	61–63	69	74								
B " " " " . . .		59	63	70	[1]	81	87	90					
M " " " " . . .		60	64	70	76	81	87	89	93	98			
W " " " " . . .			61–63	70	[1]	81	86						

A study of this table and the graphs which it summarizes shows again practically the same periodicity of endomixis in diverse races on the varied culture medium as was observed in experiment *A* of Series I. The synchronism is not quite as exact as in the former experiment but, considering all the unknown and uncontrollable variables in such a long experiment, it clearly offers further support for the conclusion that rhythms and endomixis are essentially independent of environmental conditions, and that the culture conditions merely influence initially, if at all, the appearance of endomixis, and that once established the rhythmic period characteristic of the species is maintained within rather narrow limits.

B.

This set of experiments involves a study of the periodicity of rhythms and endomixis in *different* races of *Paramecium aurelia* when bred in a relatively *small* amount of culture medium changed on *alternate days*. The cultures used, as already stated, were *As*, *Os*, *Bs* and *Ms* and their behavior with respect to the process under consideration is given in Fig. 8. A study of this graph shows that endomixis was observed in:

As at periods	59	61	65	70	[1]	0	85	90
Os " "	59	0	64	69	[1]	82	86	
Bs " "	59	0	64	70	died			
Ms " "	59	62	64	70	[1]	82	87	91

[1] The animals were not studied cytologically during this period.

Again the synchronism is very marked throughout the work, while during the initial period (59) it is perfect. The explanation of the occurrence of endomixis in every culture during the 59th period is undoubtedly due to the *marked* change of culture conditions to which these subcultures were subjected, when the experiment was initiated in period 58, by isolation of the 's'

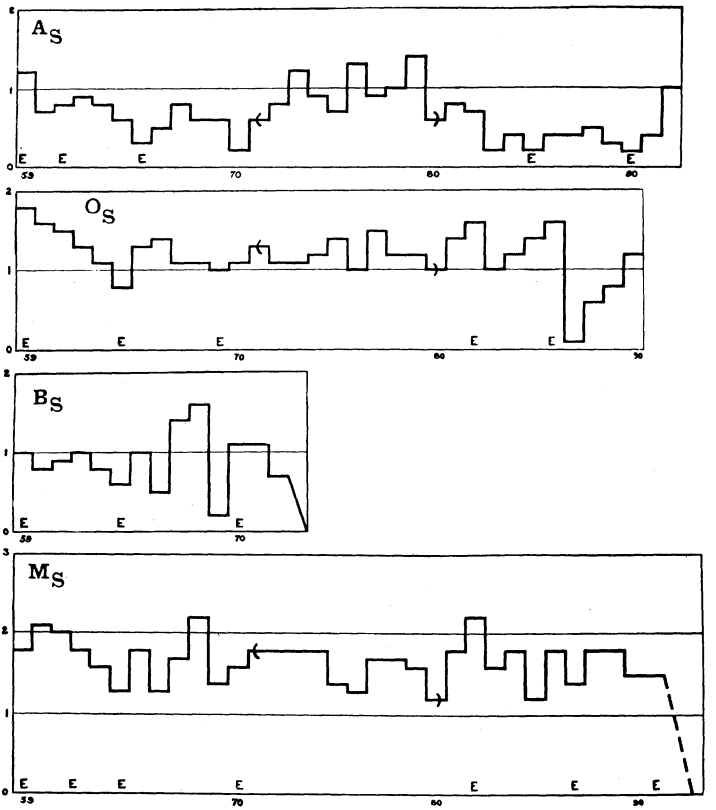

FIG. 8.

subcultures from their respective main cultures on the varied culture medium changed daily (cf. Figs. 9 to 12). This will be considered in connection with the following experiment, but obviously it is in accord with the results obtained by Woodruff and Erdmann[1] who noted in studying various *lines* of one race that the reorganization process "may occur a few days earlier in

[1] *Journal of Experimental Zoölogy*, 1914.

animals not supplied daily with fresh culture fluid than in the regular lines."

It is apparent then from this experiment that the same general conclusion which was derived from all the previous ones is again justified, viz., the culture conditions may, at most, initially influence the appearance of endomixis; but once established the rhythmic period characteristic of the species is maintained with great exactness, resulting in a remarkable synchronism of the process in the different races. General normal environmental changes obviously do not permanently alter the fundamental inherent rhythmic periods of the organism.

C.

A study now can be made of the phenomena under consideration in the *same* races of *Paramecium aurelia* when bred in a relatively large amount of culture medium supplied fresh daily, and in a relatively small amount of culture medium changed on alternate days. This involves, obviously, the comparison of the results from the two previous experiments since these were conducted simultaneously and afford the requisite data. Therefore the culture graphs of these two experiments are presented, one above the other, so that identical five-day periods coincide, in Figs. 9, 10, 11 and 12.

It is to be noted that *As* and *Os* were branched from *A* and *O* very soon after endomixis had occurred in the latter cultures; *Bs* came from *B* during the actual occurrence of endomixis; while *Ms* was branched from *M* toward the end of a rhythm as the subsequent appearance of the process in *M* at period 60 shows.

The four figures mentioned and the following tabulation of the periods in which endomixis occurred in the various subcultures shows the synchronism of the reorganization process in all the pairs of cultures under the markedly different environmental conditions. It is difficult to say whether this coincidence of the process is more exact between different races under the same culture conditions or between the same races under different culture conditions, because most of the variations are so small that they fall well within the limits of error involved in the five-day plotting method, etc. (cf. p. 440).

A at periods..	53	57	62	?	69	74	82	86	90
As " " ..		59	61	65	70	[1]	0	85	90
O " " ..		57	61	63	69	74	died		
Os " " ..		59	0	64	69	[1]	82	86	
B " " ..		59	0	63	70	[1]	81	87	90
Bs " " ..		59	0	64	70	died			
M " " ..			60	64	70	76	81	87	89
Ms " " ..		59	62	64	70	[1]	82	87	91

Thus clearly, in the long run, the *s* environment had no effect on the periodicity of the process. But, as pointed out before, endomixis appeared without exception in the four *s* cultures immediately upon their isolation from the respective parent cultures and on subjection to the stale culture fluid. Therefore, now that it is possible to compare parent and daughter lines, the obvious conclusion from the data is that endomixis was brought about earlier (except in *Bs* which was started during endomixis) by the changed cultural conditions; that is, earlier

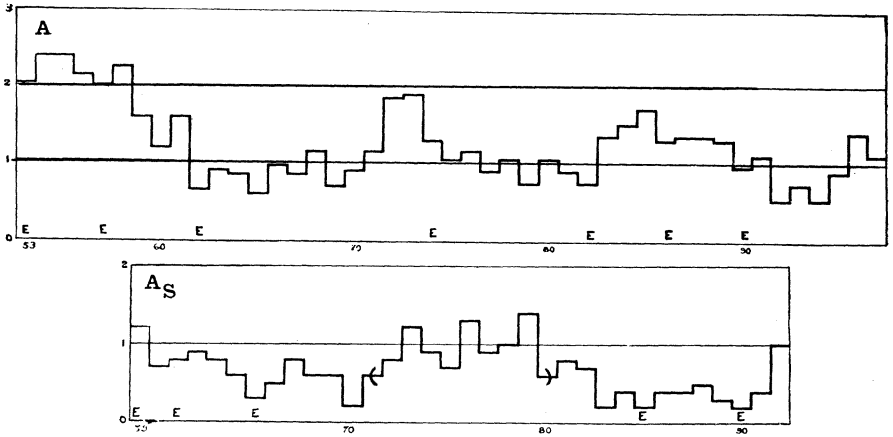

FIG. 9.

than it appeared in the parent cultures. This result, it may be noted, substantiates the conclusion from the former experiments that the remarkably exact synchronism of endomixis of various races under the most diverse environmental conditions is due to a slight initial influence on the occurrence of the process.

Figs. 9, 10, 11 and 12 show that the treatment to which the *s* cultures were subjected resulted in a distinctly lower rate of

[1] Not studied cytologically during this period.

division—on the average about three quarters of division per day lower than in the cultures subjected to the fresh culture medium, etc. This is without doubt due, as already discussed, in large part at least to the accumulated excretion products in the *s* series. But whatever the cause, the experiment affords an opportunity to study the effect of naturally changed conditions, involving a lowered fission rate, on the periodicity of rhythms and endomixis.

Now since the *s* subcultures divided at a much lower rate than the parent cultures, and since endomixis appeared fairly synchronously in parent and *s* sets, it is obvious that endomixis consistently appeared in the *s* subcultures within a smaller number of generations. In other words, the treatment of the *s*

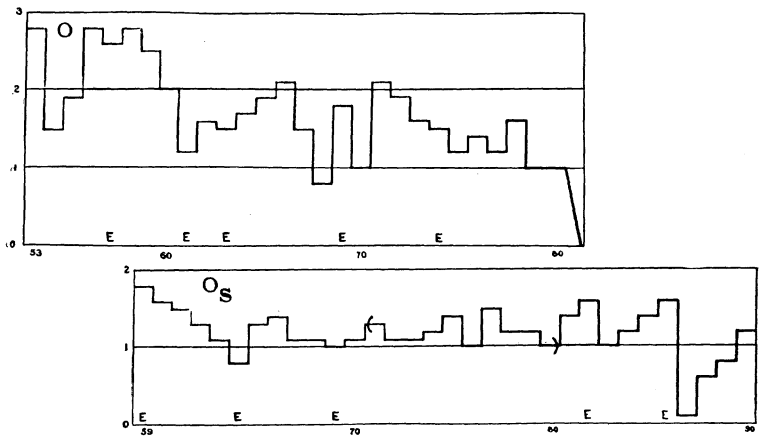

FIG. 10.

series apparently resulted merely in reducing the number of cell divisions in a given time and had practically no effect (except in the first period) on the occurrence of endomixis. On the basis of this set of cultures, then, endomixis is to a certain extent independent of the number of generations and more closely related to a time factor, if such an expression may be employed.

The *B* pair of cultures affords a fairly typical example (Fig. 11). In *B* endomixis occurred at periods 59, 63 and 70; while in *Bs* it occurred at periods 59, 64 and 70. Thus the length of

time in days between successive reorganizations is 20 and 35 in *B*, and 25 and 30 in *Bs*. Therefore in both cultures the same number of days (55) elapsed from one endomixis to its second following occurrence. On the other hand in *B* the process was in progress at the 335th, 370th and 435th generations, that is at intervals of 35 and 65 generations, while in *Bs* it took place at the 335th (when *Bs* was isolated from *B*), 360th and 380th genera-

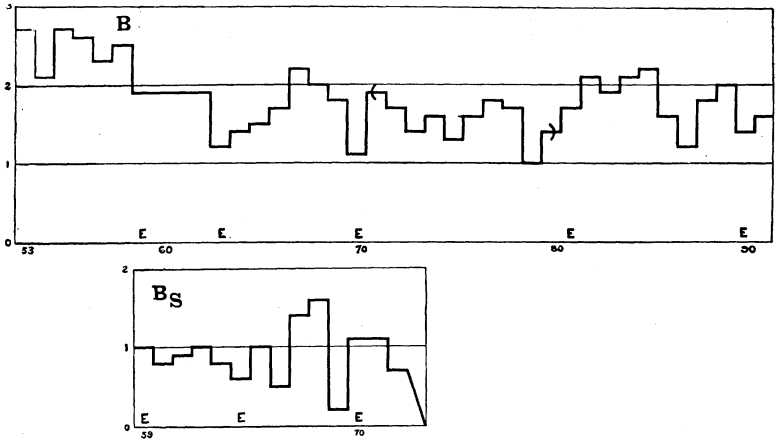

FIG. 11.

tions, or at intervals of 25 and 20 generations. Therefore in *B* 100 generations were attained from one endomictic period to the second following occurrence of the process; while in *Bs* only 45 generations occurred during the same period. To repeat: the experimental treatment apparently simply reduced the number of generations during the 100 days.

This same general result was obtained in Set *C* of Series I., though of course here the lowering of the division rate was the result of other causes. Figs. 3 and 4 show that endomixis occurred in the fourth period of the four subcultures, *A* and *At* and *AE* and *AEt*, although the rate of division and therefore the number of generations was less in *At* than in *A*, and less in *AEt* than in *AE*.

This set of experiments, then, corroborates in a clear-cut manner the general result derived from all the previous ones; that is, general environmental changes, especially if they are

pronounced, usually bring on endomixis slightly earlier than it would have occurred if the cells had been left in the environment in which they were at the last reorganization period. But after this initial change the periodicity characteristic of the organism is resumed and persists.

In addition, however, this experiment suggests another point of interest: the length of the rhythm is apparently partially

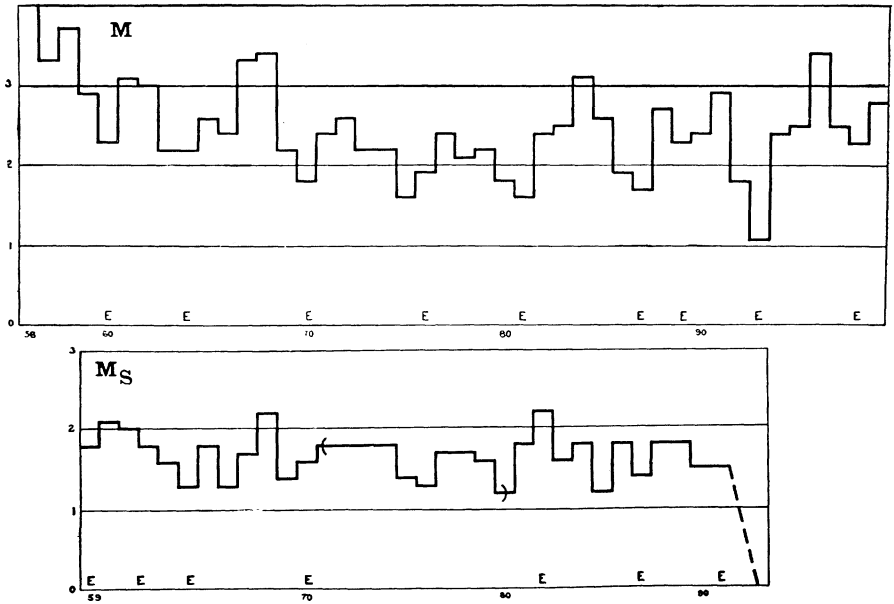

FIG. 12.

independent of the number of cell divisions—the periodicity being one in which time, so to speak, is an important factor. This is a most surprising result, because such profound reorganization phenomena as are involved in endomixis must bear a more or less definite relation to the physiological activity of the protoplasm, the best criterion of which is generally considered to be growth and reproduction as indicated by the division rate. More experiments obviously are needed to resolve this 'time factor' into its significant elements.

EXPERIMENTS—SERIES III.

Peebles states that Horlick's Malted Milk is a most satisfactory culture medium for *Paramecium* if used in a .2 per cent.

solution,[1] and therefore this seemed to offer an opportunity to study the effect of a medium radically different in composition from those previously employed in these studies. Accordingly subcultures designated *Am*1, *Am*2, *Am*3, *AEm*1, *AEm*2, *Om* and *Mm* were started from *A*, *AE*, *O* and *M* respectively, and bred on this medium. The extent of these cultures and the time of appearance of endomixis (*E*) in the two sets is shown in the following table:

Period.....	52	53	54	55	56	57	58	59	60
A.....		*E*				*E*			
*Am*1.....	(from *A*)	*E*				*E*			
*Am*2.....	(" *A*)	*E*				*E*			
*Am*3.....						(from *A*)	*E*		
AE.....					*E*			*E*	
*AEm*1.....	(from *AE*)	*E*			*E*		discontinued		
*AEm*2.....						(from *AE*)	*E*		
O.....						*E*			
Om.....	(from *O*)					*E*			
M.....									*E*
Mm.....						(from *M*)	*E*		

This table shows that the malted milk medium did not change at all the periodicity of endomixis in either the *A* or *O* milk subcultures, while it consistently brought it about earlier in the *AE* and *M* milk series. The results from the *AE* and *M* cultures are therefore in accord with the results obtained in Series II of these experiments, while those with *A* and *O* apparently differ. However, the non-appearance of endomixis earlier in the *Am* series is readily explained by the fact that the process was just about to occur in the parent culture when the *Am* subcultures were branched, as is shown by the fact that it appeared in *A* during the following period.

The experiments with malted milk are obviously not extensive enough to give any details of the effect of long subjection to this medium on endomictic periodicity, but they adequately answer, it is believed, the purpose of the present study by again indicating that the appearance of endomixis may be slightly

[1] Peebles, "Regeneration and Regulation in *Paramecium caudatum*," BIOL. BULL., 1912.

altered temporarily by subjecting *Paramecium* to a markedly changed environment.

Experiments—Series IV.

This series is a brief repetition of Series I., since it comprises a comparison of the periodicity of endomixis both in the same races when bred under varied and constant culture conditions, and in different races bred under varied and constant culture conditions. The cultures employed were *A*, *AE* and *B* which were used in the first series, and also two other cultures, *M* and *W*.

The following table shows a practically perfect synchronism of endomixis in all the races under the different environmental conditions:

Period.....	81	82	83	86	87	88	89	90
A.....		*E*		*E*				*E*
At2.....			(from *A*)	*E*				*E*
AE.....		*E*		*E*				*E*
AEt2.....			(from *AE*)	*E*				*E*
B.....	*E*				*E*			*E*
Bt2.....			(from *B*)	*E*				*E*
M.....	*E*				*E*		*E*	
Mt2.....			(from *M*)	*E*				*E*
W.....	*E*			*E*				
Wt2.....			(from *W*)	*E*				

This is exactly the same result which was obtained in the experiments of Series I. In Series I this synchronism was most satisfactorily accounted for by assuming that there was an initial effect on the periodicity by the change of environmental conditions to which all the cultures were subjected at the start. That this assumption was justified is shown by experiment *C*, Series II (cf. p. 455). However, it is to be noted in the present experiment that there is no apparent initial influence of the changed culture conditions, but this is probably due to the fact that the *A*, *AE*, *B*, *M* and *W* cultures had been so long under the same environmental conditions before their respective *t2* subcultures were branched from them. Consequently the syn-

chronism of the *t2* set is due merely to maintaining the periodicity of the respective parent cultures.

General Summary.

All four series of experiments show that the general 'time-periodicity' of rhythms and endomixis in *Paramecium aurelia* is the same in the several races which have been studied under the following environmental conditions:

1. Varied culture medium changed daily, and at room temperatures.
2. Varied culture medium changed on alternate days, and at room temperatures.
3. Constant beef extract culture medium and at a temperature of 26° C.
4. Horlick's malted milk medium, and at room temperatures.

Thus it seems clear that one question which this study was planned to elucidate has been answered: General changes in the environment of the animals, as markedly different culture media and temperatures, such as may be termed normal changes, do not permanently modify the length of the rhythm or the time between successive endomictic periods which is characteristic of the species.

However, sudden marked changes in normal culture conditions may initially induce the appearance of the definitive endomictic phenomena slightly earlier than they would have occurred if the cell had been continued under its former environmental conditions; but this initial disturbance is soon compensated for, usually within the present rhythm, so that the previous characteristic periodicity is again resumed.

Throughout all the work there is evident a remarkable *synchronism* of the endomictic process in all the races bred simultaneously, regardless of the environmental conditions. Thus not only is the periodicity of endomixis, or length of the rhythm, the same, as stated above, but also the rhythmic periods are synchronous. The explanation of this is clearly due, in the experiments involving the most marked changes in the cultural conditions, to an initial effect of these changes, which brings into line, so to speak, the appearance of endomixis in all the cultures.

Consequently it is highly probable that a slight initial shift of the definitive onset of endomixis in the various races is the explanation of the nearly simultaneous appearance of the process in all the races under all the conditions.

Although the 'time-periodicity' characteristic of the species has been shown by the present experiments to be practically unmodifiable under the general environmental changes which were employed, it has been found that the 'generation-periodicity'—or the number of cell divisions between one occurrence of endomixis and the next—may be modified to a considerable degree by the culture conditions which lower the division rate. In other words, the rhythm appears to be more susceptible of modification in regard to generations than time. As has been previously noted, this is a surprising result, since a profound reorganization process such as endomixis must be closely related to the general metabolism of the cell and this is expressed to a large extent in growth and reproduction. Further work on this problem is in progress.

Finally, the cessation of endomixis in these experiments was invariably followed, usually within a rhythm or two, by the death of the culture involved. This indicates strongly, if it does not prove, that a periodic occurrence of the definitive endomictic phenomena is a *sine qua non* for the continued life of the race—a conclusion which is concordant with all previous data in regard to endomixis.